International Journal of Agriculture, Environment and Bioresearch

Vol. 3, No. 03; 2018

ISSN: 2456-8643

USE OF TESTICULAR STEROIDS IN TILAPIA MANAGEMENT FOR IMPROVED AQUACULTURE PRODUCTIVITY

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ABSTRACT

The need to find natural replacements for synthetic hormonal anabolic steroids for use in livestock production has received considerable attention in the recent past. The present study was, therefore, designed to assess the efficacy of using testicular tissue or fluid on the development of Tilapia fish species (Oreochromis andersonii). In the first of two experiments, a 48% crude protein fry diet was formulated to contain 50% testicular tissue powder and 50% commercial fry diet. Fry were fed either twice (n=50), thrice (n=50) or four (n=50) times daily for 28 days. For an additional 32 days, fry were fed commercial fry feed. Mean weight, Standard length and total length of fry were not affected by feeding fry with testicular tissue. Similarly, increasing the frequency of feeding did not influence fry weight, standard or total length. In the second experiment, fry were fed a diet that contained steroid-free testicular fluid or feed that contained neat testicular fluid. The 45% crude protein ration was given to O. andersonii twice daily for 28 days. Fry were fed a commercial fry ration for an additional 32 days. Testicular fluid inclusion significantly affected final weight (0.12±0.005g vs 0.099±0.004g, P=0.002), total length (1.91±0.03cm vs 1.81±0.03cm, P=0.02), and standard length (1.50±0.03cm vs 1.39±0.02cm, P=0.003). It is concluded that use of testicular fluid extract is a convenient tool that enhances fish fry performance and may have potential for stimulating improved fry and fish growth to improve aquaculture productivity especially among smallholder farmers.

Keywords: performance steroids testicular tilapia

1. INTRODUCTION

In the quest to meet the human need for animal protein in his diet aquaculture has, in the recent past, made significant contribution to that need. It is predicted that by the year 2020, nearly 41 % of the world's fish production will owe its origin from the aquaculture industry (Krishen et al., 2009). Reports indicate that Tilapine species are widely grown worldwide because of their tolerance to harsh environmental conditions such as temperature changes, high salinity, poor water quality, tolerance of high stocking density, and rapid growth (Maluwa and Brooks, 1996; Corpei, 2001). Additionally, they are reported to thrive on a wide range of herbivorous and omnivorous feeds and resistance to disease challenge (Beveridge and McAndrew, 2000). These attributes complement the fact that Tilapia have high protein content, large size, breed rather easily, good utilization of artificial diets, rapid growth rate [taking only 6 to 7 months to grow to harvest size], and their excellent quality of flesh that is generally appetizing to consumers.

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Consequently, a number of Tilapine cichlids have been part of some major aquaculture developmental efforts.

In order to spur on this need for increased production, Tilapine farming has seen considerable and rapid industrialisation. Terrestrial animal byproducts including poultry by-product meal, blood meal, meat and bone meal have been widely used as protein sources for many fish species, due to their high protein content and good essential amino acid content which accounts for about 50% of fish feed (Tacon, 1993). To promote growth, heavy focus is on ensuring feeds have high protein content. Protein sources include fishmeal, blood meal, and bone meal have been used but this has instead led to higher costs.

The predicament caused by increased high feeding and protein costs created demand for growth promoters as cheaper means of production and use of these products has steadily been on the rise (Efnen *et al.*, 2013). Considerable information is available on the growth promoting efficiency of anabolic steroid hormones (Nakamura and Takahashi, 1985; Tayamen and Shelton, 1978; Goudi *et al*., 1983; Desprez *et al*., 2003), mainly in the form of 17α - methyltestosterone [MT], in fishes (McBride, 1973; Macintosh, 2005). Such synthetic hormones closely mimic the naturally-produced hormone testosterone and, consequently, this and other synthetic forms of testosterone have been used widely as hormones administered to replace or supplement natural testosterone (Macintosh, 2005) used as growth promoters and tools for sex reversal in fish.

Despite the promise of better productivity emanating from use of anabolic steroids, there are reports of increased risks of long term exposure of workers handling MT during food preparation and feeding which may cause adverse effects on their health (Green *et al.*, 2000). There have also been reports that hormones in the form of either active metabolites excreted by the treated fish or leachates from uneaten food can build up in a closed water systems (Abucay and Mair, 1997) with potential for adverse effects on untargeted elements of the pond ecosystem as well.

As a consequence of these concerns, alternative natural sources of testosterone such as animal testicles and plants such as *Tribulus* terrestris have been considered (Ghosal, & Chakraborty, 2014). Indeed testes have been used in various forms of whole goat, sheep, bull or pig testes used after either freeze- or oven drying prior to incorporation into feed. Although the anabolic effects arising from use of testes has been demonstrated, the precise mechanism by which this happens remains undetermined. This view is consistent with Sulieman *et al.*, (2012) who found growth rate of Nile Tilapia to increase with increase in the level of testicular tissue included in the diet. It is plausible that improvement in growth performance may be attributed to the extra protein from the testes tissue or due to the steroid content of the testes. In order to facilitate better understanding and use of these natural steroids to spur better aquaculture productivity, it is imperative that this confounding set of results is resolved.

Therefore, the objective of this study was to assess the efficacy of using testicular tissue or fluid on the development of Tilapia fish species (*Oreochromis andersonii*).

2. MATERIALS AND METHODS

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In order to prepare testis powder for the first experiment, ten intact testes (4.6kg) from mature pigs were obtained from a local slaughter. After slicing, the testes were oven dried at 90^{O}C for 6 days. Dried testes tissue was grated and sieved to obtain a fine meal. Experimental diets were prepared by incorporating the testes meal powder at a ratio of 1:1 with commercial fish ration (26% CP) to yield a test diet that had 48.1% CP for a period of 28 days. Control fry were given a commercial fish meal. Fry were fed at 20% of their body weight daily either twice (T2), thrice (T3), or four (T4) times each day. After the first 28 days, all fry were fed the same isoprotein diet made of commercial meal (35%) and fishmeal (65%) for a period of 32 days. A sample of fry were used to obtain the initial weight (Wt), standard (SL) and total length (TL). After the experimental period all fry were weighed and their standard and total length were measured.

For the second experiment, another ten testes (4.6kg) were skinned and the testicular fluid was obtained using the testis fine needle aspiration with mapping technique. Briefly, a pointed-edge of a surgical blade was used to puncture the testes at different locations until the entire surface was covered with punctures through which testicular fluid was aspirated. The aspirated fluid (500ml) and was separated into two portions. Testicular fluid was filtered and either used as it or stripped of its androgen content by sieving it over activated charcoal twice.

Commercially available fish feed pellets (26% CP,) and fishmeal (65% CP) were mixed in proportions of 35% and 65%, respectively, to yield a 45% diet was used in the second experiment. Both the pellets and the fishmeal were ground into powder and sieved prior to use in the above formulations. Neat testicular fluid was added to one half of the feed and thoroughly mixed while to second half, steroid-free testicular fluid was added and thoroughly mixed. Both feed samples were then oven dried for two days at 90^{O}C, allowed to cool, ground, sieved and then stored for later use. Fry were fed these diets twice daily for a period of 28 days after which the non-steroid-stripped diet of fish feed pellets (26% CP,) and fishmeal (65% CP) mixed in proportions of 35% and 65%, respectively was used for further 30 days. A sample of fry were used to obtain the initial weight (Wt), standard (SL) and total length (TL). At the end of the experimental period, all fry were weighed and their standard and total length were measured.

For both experiments, perspex glass aquaria measuring 27cm width, 90cm length, and 30cm height and equipped with an aerator and tubing for providing air, were used to raise five-day-old Tilapia fry (*Oreochromis andersonii*) that were obtained from a commercial hatchery. The aquaria were divided into two groups of three aquaria each at a stocking rate of 25 fry per aquarium. Water was changed once each week from inception until the end of the experiment.

Other experimental data was also derived by computations from the initial data collected on all fry. These included the following;

1. Mean weight gain (MWG) = Final mean weight of fry – initial weight of fry in grammes (g).
2. Specific growth rate (SGR) was determined as described by Adewolu (2008) thus SGR=100($\frac{Ln\,Wt2 - Ln\,Wt1}{T2 - T1}$)

Where W1 = the initial weight, Wt2 = the final weight, Ln Wt1 = Natural logarithm of the initial weight, Ln Wt2 = natural logarithm of the final weight of fry, T1 = starting date and T2 = final date of the experiment. The weights are grammes (g) while the lengths are centimetres (cm).

3. Condition factor (K) = 100 ($\frac{W}{SL3}$) derived after Charo-Karisa *et al.*, (2006), where W = weight of fry in grammes (g), and SL = standard length of fry in centimetres (cm).

Experimental data was subjected to either a one-way analysis of variance (ANOVA, experiment one) or Student's test (t-test) using the General Linear Model (GLM) Procedures of the Statistical Analysis Software (SAS, 2004) at 5% (*P=0.05*) significance level. Where main effects were significantly different, treatment means were separated using the Duncan's multiple range test, as appropriate.

Data are presented as mean ± standard error or the mean (SEM). There were no differences between the feeding frequencies used in this study with regard to mean weights (Figure 1), total length (Figure 2) and standard length (Figure 3) for fry fed twice, thrice, of four times daily.

The condition factor for fry fed was similar (0.003) for the frequencies of feeding fry in this experiment. Similarly, the mean weight gain for twice, thrice and four times of feeding were 0.08g, 0.06g and 0.08g, respectively. Additionally, the mean SGR for the same groups were 3.30%, 2.81%, and 3.31% per day.

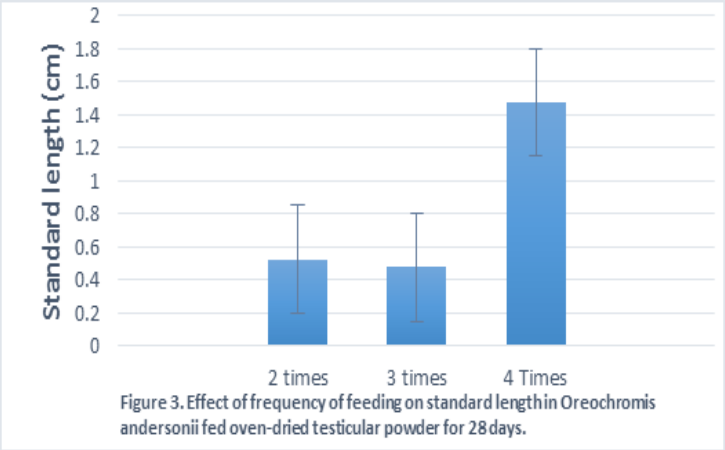

Figure 3. Effect of frequency of feeding on standard length in Oreochromis andersonii fed oven-dried testicular powder for 28 days.

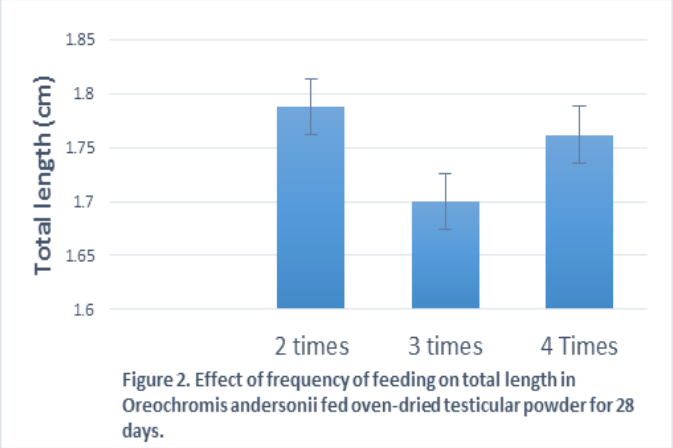

Figure 2. Effect of frequency of feeding on total length in Oreochromis andersonii fed oven-dried testicular powder for 28 days.

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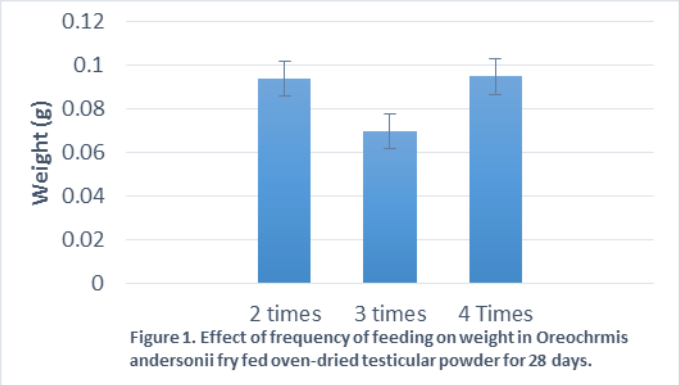

Figure 1. Effect of frequency of feeding on weight in Oreochrmis andersonii fry fed oven-dried testicular powder for 28 days.

Data are presented as least square means (LSM) ± standard error of the mean (SEM). There were significant differences (*P=0.006*) in final weights attained by the fry in given feed with steroid and those given feed denuded of its steroid content (Table 1). Similarly, the natural steroids contained in testicular fluid resulted in higher total (*P=0.04*) and standard (*P=0.007*) lengths among Tilapia fry

Table 1. Mean weight (Wt), standard (SL), and total (TL) length among Tilapia (*Oreochromis andersonii*) fry given feed either with natural testicular fluid steroid or testicular fluid from which steroids were removed.

Parameter	Treatment		Prob. (*P*) level (*M1=M2*)
	Steroid	Steroid Stripped	
Weight (g)	0.12 ± 0.004^{a}	0.10 ± 0.004^{b}	*P=0.006*
Standard length (cm)	1.5 ± 0.02^{a}	1.4 ± 0.02^{b}	*P=0.007*
Total length (cm)	1.91 ± 0.03^{a}	1.82 ± 0.03^{b}	*P=0.04*

Means within a row that have different letter superscripts are significantly different (P<0.01).

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The condition factor for fry that were given steroid-containing feed and for that whose feed did not contain any steroid were 0.002g cm-3 and 0.003g cm-3, respectively. Similarly, the mean weight gain was 0.053g and 0.033g, in the same order. Additionally, the mean specific growth rate for the two treatments was 0.41% and 0.28 % per day.

3. DISCUSSION

It was the intension of this study to assess and confirm the anabolic capacity contained in boar testes when the frequency of such feeding is increased from the recommended and more common rate of twice daily. However, the values obtained in this study showed no improvement when that frequency was increased beyond the norm. In terms of its effect on mean weight gain and standard length, there was a trend for better performance with the four times a day feeding followed by those fed twice a day. Our current results are in conformity with those reported recently (Sulieman et al., 2013) when Oreochromis niloticus fry were fed dried bulls testes at 50% inclusion rate given three times daily. These findings are in contrast to those reported by Phelps et al., (1996) whose four-times a day fed fry attained a much higher weights in less time. While it is not clear why these discrepancies were noted, it is plausible that the rearing conditions contributed to the lower rates observed in our study partly supported by a higher than normal mortality rate that was experienced. This notwithstanding, use of 50% goat testes meal given ad libitum (Odin et al., 2011) resulted in total length measurements similar to the fry fed four times a day in our study. The performance of the fry with regard to efficiency of utilizing feed was also very poor. This finding was at variance with results obtained by Sulieman et al., (2013) who reported a six-fold improved feed utilization efficiency. Such variations in our findings were further attributed to low water temperature (below 20oC) and the frequent changing of water in the aquaria. It is expected that at low temperatures, fish fry perform poorly because this leads to, inter alia, reduced feed intake (Mjoun, Rosentrater, and Brown, 2010) and resultant compromised growth.

In our attempt to demonstrate that it is better to use only testicular fluid, rather than testicular tissue, as a source of the androgen testosterone, we used testicular fluid which was shown to contain the necessary anabolic steroid. The Oreochromis andersonii fry that were fed on androgen (testosterone)-containing fluid had significantly higher weights when compared to fry that received feed with androgen-stripped testicular fluid. These results are consistent with those reported by Sulieman et al., (2012), whose fry had a higher growth rate which increased with increase in inclusion level of testicular tissue. The use of androgen stripped testicular fluid demonstrated that indeed testes, and the fluid drained from it, do contain an anabolic steroid. This should negate any need to use whole testes when compounding feed for growth promotion in livestock. Use of whole testicular tissue creates an unnecessary handicap of dealing with the animal protein and fats that the tissue contains and may make ration formulation more complicated than it need be. In this case, use of testicular fluid poses less manipulation problems during ration formulation and fry feed storage post formulation.

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The condition factor in the second experiment of this study was higher in androgens-treated fry than that of androgen-stripped group. Although the absolute values were much lower than those reported previously (Anene, 2005, Mahomoud et al., 2011), they were indicative of better utilization of feed resources by the fry fed androgens-containing feed. Furthermore, the feed conversion ratio also varied in similar manner. While the benefit derived from such usage was in agreement with those reported by Sulieman et al., (2012), our study shows that it may not necessarily be the added dietary proteins that predisposed the improved performance. The improvement, in that and this study, could merely be due to the anabolic steroid contained in testicular fluids. It is our considered view that using fluid facilitates a more uniform mixing of the feed.

It is concluded, therefore, that use of natural steroids contained in testicular fluid improved Oreochromis andersonii performance. It is also that recommended that the said natural steroids that are in abundance in various plant and animal organs such as testes, should be further explored as a growth promoter in aquaculture production.

ACKNOWLEDGEMENTS

The authors are grateful to staff at the Department of Fisheries in the Ministry of Fisheries and Livestock Development for technical guidance on fry management. Thanks are also extended to the Animal Nutrition laboratory for assistance with the feed analysis and aquaria management. We are also indebted to the Department of Biological Sciences for providing the activated charcoal.

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